201-15138

March 10, 2004

Administrator
US EPA
P.O. Box 1473
Merrifield, VA 22116
Attn: Chemical Right-to-Know Program

Dear Administrator:

On behalf of the member companies of the HPV Committee, the International Association of Color Manufacturers is pleased to submit the test plan and robust summaries for Sunset Yellow (FD&C Yellow 6). The IACM HPV Committee has chosen not to belong to the HPV Tracker System for submission of test plans and robust summaries. We are therefore submitting the test plan and accompanying robust summaries directly to EPA to make available to the public. A hard copy of this submission is available upon request. The EPA registration number for the IACM HPV Committee is 201-12671.

Please feel free to contact me with any questions or comments you might have concerning the submission (tadams@therobertsgroup.net or 202-331-2325).

Sincerely,

Timothy Adams, Ph.D.
Technical Contact Person for IACM HPV

201-15138A

Test Plan for
Sunset Yellow
CAS No. 2783-94-0

Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:
The International Association of Color Manufacturers/HPV Committee
1620 I Street, NW, Suite 925
Washington, DC 20006
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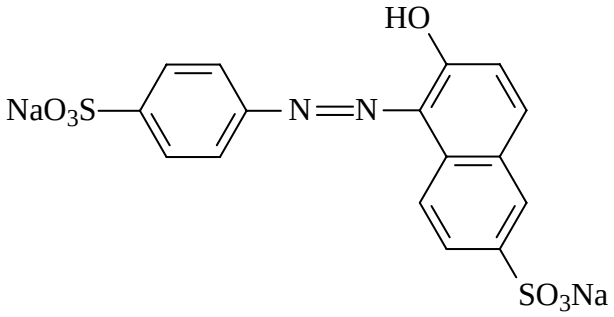
Test Plan for

Sunset Yellow

1 IDENTITY OF SUBSTANCES



Sunset Yellow

CAS No. 2783-94-0

Synonyms:
FD&C Yellow 6
C.I. Food Yellow 3

2 CATEGORY ANALYSIS

2.1 INTRODUCTION

The International Association of Color Manufacturers (IACM) has volunteered to participate in the EPA's Chemical "Right-to-Know" Program. IACM is committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and, where needed, conducting additional testing on the chemicals used by the color industry in order to assure their human and environmental safety. The category analysis, test plan, and robust summaries represent the first phase of IACM's commitment to the Chemical "Right-to-Know" Program.

2.2 BACKGROUND INFORMATION

This test plan provides data for FD&C Yellow 6 (Sunset Yellow). FD&C Yellow No. 6 is a yellow powder that is freely soluble in water and is used as a food colorant in diary products, snack foods, cereals, bakery items, confectionery products, frozen deserts and beverages, cosmetics, ingested and externally applied drugs, and dietary supplements.

FD&C Yellow No. 6 is an azo dye. Azo compounds are formed from arenediazonium ions reacting with highly reactive aromatic compounds, in what is called a diazo coupling reaction. Azo compounds are generally deeply colored because the azo linkage brings the two aromatic rings into conjugation [Solomon, 1996]. In addition to possessing extended conjugation, many azo dyes are also ring substituted with sulfonic acid substituents, which significantly increase polarity and water solubility and decrease absorption *in vivo*.

2.3 REGULATORY STATUS

FD&C Yellow 6 is a certified color additive approved in the United States to color food, drugs and cosmetics. Certified color additives are synthetic organic compounds that must meet high purity specifications established by the Food and Drug Administration (FDA) (see Table 1 below). Each batch of manufactured certified color in the United States is

tested by the FDA for compliance with these specifications [Frick and Meggos, 1988]. Certified color additives are among the most thoroughly studied of all food ingredients because of the rigorous testing for human health endpoints required by the 1960 Color Additive Amendments to the FD&C Act [Hallagan, 1991]. There are currently only seven certified color additives approved for food, drug and cosmetic use in the United States.

Table 1. US FDA Specifications

FD&C Yellow No. 6 shall conform to the following specifications and shall be free from impurities other than those named to the extent that such other impurities may be avoided by good manufacturing practice (21 CFR 74.706)

- Sum of volatile matter at 135°C (275°F) and chlorides and sulfates (calculated as sodium salts), not more than 13 percent.
- Water-insoluble matter, not more than 0.2 percent.
- Sodium salt of 4-aminobenzenesulfonic acid, not more than 0.2 percent.
- Sodium salt of 6-hydroxy-2-naphthalenesulfonic acid, not more than 0.3 percent.
- Disodium salt of 4,4'-(1-triazene-1,3-diyl)bis[benzenesulfonic acid], not more than 0.1 percent.
- Sum of the sodium salt of 6-hydroxy-5-(phenylazo)-2-naphthalenesulfonic acid, not more than 1 percent.
- Sum of the trisodium salt of 3-hydroxy-4-[(4-sulfophenyl)azo]-,7-naphthalenedisulfonic acid and other higher sulfonated subsidiaries, not more than 5 percent.
- 4-Aminoazobenzene, not more than 50 parts per billion.
- 4-Aminophenyl, not more than 15 parts per billion.
- Aniline, not more than 250 parts per billion
- Azobenzene, not more than 200 parts per billion.
- Benzidine, not more than 1 part per billion.
- 1,3-Diphenyltriazene, not more than 40 parts per billion.
- 1-(Phenylazo)-2-naphthalenol, not more than 10 parts per million.
- Lead (as Pb), not more than 10 parts per million.
- Arsenic (as As), not more than 3 parts per million.
- Mercury (as Hg), not more than 1 part per million.
- Total color, not less than 87 percent.

FD&C Yellow No. 6 was first listed for food use in the United States in 1929. In 1994, 994,406 kg of FD&C Yellow No. 6 dye and 283,680 kg of FD&C Yellow No. 6 lake were certified for use in the United States.

The World Health Organization/Food and Agriculture Organization Joint Expert Committee for the Evaluation of Food Additives (WHO/FAO JECFA) have also evaluated the safety of FD&C Yellow No. 6 used as a coloring agent in food. An average daily intake (ADI) of 0-2.5 mg/kg bw/day was assigned by JECFA in 1982 based on the extensive human toxicological information available (see Table 2 below).

Table 2. Regulatory Approvals/Consumption Limits[1]

USA	FD&C Yellow No. 6 may be safely used for coloring foods (including dietary supplements) generally in amounts consistent with good manufacturing practice, except that it may not be used to color foods for which standards of identify have been promulgated under section 401 of the act unless added color is authorized by such standards (21 CFR 74.706).
EEC	0-2.5 mg/kg (14th series, 1983)
JECFA	0-2.5 mg/kg (26th report, 1982)

Based on the long history of use of FD&C Yellow No. 6 in food, the many hazard assessments performed by the United States FDA and WHO/FAO JECFA, and the current regulatory status of FD&C Yellow No. 6, there is no compelling evidence that this substance should be further tested for human health endpoints in the EPA Chemical "Right to Know" Program.

2.4 STRUCTURAL CLASSIFICATION

FD&C Yellow No. 6 is principally the disodium salt of 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonic acid. The trisodium salt of 3-hydroxy-4[(4-sulfophenyl)azo]-2,7-naphthalenesulfonic acid may be added in smaller amounts (USFDA-21 CFR 74.706). The diazo nucleus (-N=N-) contains a benzene ring substituted with a *p*-sulfonic acid group and a naphthalene ring substituted with *o*-hydroxy and *p*'-sulfonic acid groups.

[1] IACM, 2003

2.5 INDUSTRIAL PRODUCTION

FD&C Yellow No. 6 is manufactured by coupling diazotized sulfanilic acid with 2-napthol-6-sulfonic acid. The dye is isolated as the sodium salt and dried.

2.6 PHARMACOKINETICS AND METABOLISM

The major route of metabolism for FD&C Yellow No. 6 is bacterial azo reduction in the gut. The major metabolites of FD&C Yellow No. 6 are sulfanilic acid and amino-2-naphthol-6-sulfonic acid [Honohan *et al*., 1977].

Rats orally administered a single oral dose of 100 mg FD&C Yellow No. 6 (Sunset Yellow) excreted 0.8% of the intact dye in the feces [Radomski & Mellnger, 1962]. (^{14}C) Sunset yellow (labeled on C-8 of naphthalenic moiety) was orally administered to female rats, and urine and bile were collected. After 96 hours, 8.5% of the 1-amino-2-naphthol-6-sulfonic acid equivalent, 37.4% of the sulfonic acid equivalent and 0.3% of intact dye were excreted in the urine; biliary excretion of sunset yellow was 1.5% [Honohan *et al*., 1976]. In another study, female Simonsen/Sprague-Dawley rats orally administered 1 ml of an aqueous solution containing 2-25 mg of Sunset Yellow excreted 0.3 and 1.5% of the intact dye in the urine and bile, respectively, and 37% of the sulphanilic acid equivalents in the urine. More than 90% of the dye was excreted in the feces [Honohan *et al*., 1977].

3 TEST PLAN

3.1 CHEMICAL AND PHYSICAL PROPERTIES

3.1.1 Melting Point

FD&C Yellow No. 6 is a solid and decomposed without melting when heated to 390 °C [NTP, 1981]. Accordingly, the melting point of FD&C Yellow No. 6 was calculated to be 350 °C using modeling software [MPBPVPWIN EPI Suite, 2000].

3.1.2 Boiling Point

The boiling point of FD&C Yellow No. 6 was calculated to be 837 °C [MPBPVPWIN EPI Suite, 2000]. Technically, data for this endpoint are not required given that this material is a solid and would likely decompose upon heating to elevated temperatures.

3.1.3 Vapor Pressure

The calculated vapor pressure for FD&C Yellow No. 6 has been reported to be 1.43 X 10-22 mm Hg at 25°C [MPBPVPWIN EPI Suite, 2000]. Given the high molecular mass of FD&C Yellow No. 6 (452.37) and the estimated Henry's law constant for azo dyes of 10^{-15} atm-m^3/mol it is highly unlikely that FD&C Yellow No. 6 would exhibit any significant (less than 0.001 mm Hg) vapor pressure. This is predicted by the MPBPVPWIN model. Based on these data, the vapor pressure is less than 1 X 10^{-20} mm Hg.

3.1.4 Octanol/Water Partition Coefficients

Log K_{OW} value for FD&C Yellow No. 6 is –1.18 [KOWWIN EPI Suite, 2000]. The experimental log K_{OW} value would be difficult to obtain by OECD methods given the large difference between water solubility and anticipated solubility in octanol. Based on the observations that FD&C Yellow No. 6 is freely water soluble (190,000 mg/L) and

essentially insoluble in a relatively polar solvent like ethanol (10 mg/L) (Marmion, 1991, robust summary not included), it is anticipated that the log K_{OW} value for this substances would exceed 6.0.

3.1.5 Water Solubility

FD&C Yellow No. 6 has a reported water solubility of 190,000 mg/L at 2°C, 190,000 mg/L at 25 °C, and 200,000 mg/L at 60 °C [Marmion, 1991]. The solubility of FD&C Yellow No. 6 in 100% glycerol is 200,000 mg/L at 25 °C while the solubility in ethanol is reported to be 10 mg/L at 60 °C (Marmion, 1991, robust summary not included). The solubility of FD&C Yellow No. 6 in octanol is expected to be less than 1 mg/L.

3.1.6 New Testing Required

None.

3.2 ENVIRONMENTAL FATE AND PATHWAYS

3.2.1 Photodegradation

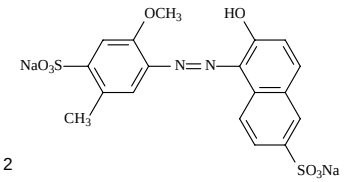
Direct and indirect photolysis experiments were conducted on the structurally related monoazo dye, FD&C Red No. 40[2], using two 15-watt low pressure lamps as the ultraviolet light source. Following 50 minutes of exposure to the lamps, FD&C Red No. 40 concentration decreased by 7% in the direct experiment. In the indirect experiment which used acetone as the sensitizer, the concentration of FD&C Red No. 40 decreased by 99% after 20 minutes [Pasin and Rickbaugh, 1991]. The calculated half-life for FD&C Yellow No. 6 in hydroxyl radical reactions is 31.9 hours [AOPWIN EPI Suite, 2000].

3.2.2 Stability In Water

FD&C Yellow No. 6 does not contain functional groups (*e.g.*, esters, amides, acetals, epoxides, lactones, *etc*.) that hydrolyze in water. The only potential reactivity in water would involve desulfonation of the aromatic sulfonic acid or its corresponding sulfonic acid salt. In aqueous acid (sulfuric acid), aromatic sulfonic acids desulfonate at temperatures of 100 to 175 ^{o}C. These conditions would not typically be encountered in the environment. Therefore, FD&C Yellow No. 6 and its corresponding salts are anticipated to be stable in water.

3.2.3 Biodegradation

The biodegradability of azo dyes substituted with a phenolic OH and two sulfonic acid groups consistently show that these substances are not absorbed onto activated sludge and, therefore, are not biodegradable [Shaul *et al.*, 1990]. Incubation of 1.0 or 5.0 mg/L of a structurally related azo dye, (1-naphthalenesulfonic acid, 4-hydroxy-3-[(4-sulfo-1-



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naphthalenyl)azo]-, disodium salt)[3] with activated sludge from a sewage treatment plant revealed that the concentration of dye remained essentially constant in the influent flow, primary effluent, and activated sludge effluent. Essentially no azo dye was absorbed by activated sludge. Two other azo dyes ring-substituted with sulfonic acid groups (Acid Orange No. 10 and Acid Red No. 1) exhibited a similar behavior in these experiments.

FD&C Yellow No. 6 was not predicted to be readily degradable by BIOWIN model calculations [BIOWIN EPI Suite, 2000].

3.2.4 Fugacity

Transport and distribution in the environment were modeled using Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70 [EPIWIN EPI Suite, 2000]. The principal input parameters into the model are molecular weight, melting point, vapor pressure, water solubility, and log K_{OW}.

As expected, the model predicts that FD&C Yellow No. 6 is distributed completely to the water and soil compartments. Consistent with the extremely high water solubility and low log K_{OW} data, FD&C Yellow No. 6 showed no distribution to the fish compartment. These data are consistent with ecotoxicity data for aromatic sulfonic acid derivatives that demonstrate essentially no absorption and toxicity to fish even at concentrations exceeding 1000 mg/L.

3.2.5 New Testing Required

None.

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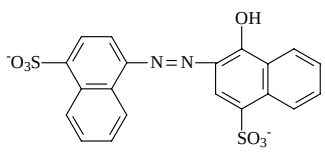
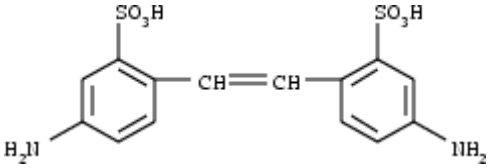
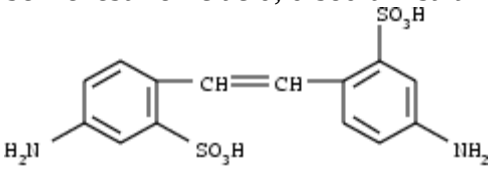
3 

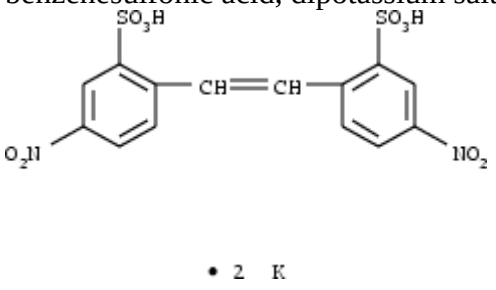
3.3 ECOTOXICITY

3.3.1 Acute Toxicity to Fish

Based on input parameters for molecular weight (452.37), water solubility (190,000 mg/L at 25 °C), and melting point (390 °C), the calculated 96-hour LC50 for FD&C Yellow No. 6 is 6,044 mg/L [ECOSAR EPI Suite, 2000] indicating a very low order of acute toxicity. The extensive water solubility and limited lipophilicity of FD&C Yellow No. 6 is to a large extent, a function of the presence of aromatic sulfonic acid and phenolic ring substituents. The extensive studies on the ecotoxicity of aromatic sulfonic acids indicate a very low order of toxicity to fish [Greim *et al.*, 1994]. Experimental LC50 values are available for stilbene sulfonic acids in which the N atom in the diazo dye is replaced by C. As indicated in Table 3 below, acute fish toxicity studies on salts of stilbene sulfonic acid derivatives result in a 96-hour LC50 value greater than 10,000 mg/L. Also, 48-hour and 72-hour LC50 concentrations of 200 and greater than 1000 mg/L, respectively have been reported [Greim *et al.*, 1994]. These values are consistent with calculated values.

Table 3

Name	Acute Toxicity to fish
2,2’-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid 	48-hour LC50: 200 mg/L
2,2’-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, disodium salt  • 2 Na	72-hour LC50: greater than 1000 mg/L

2,2’-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, dipotassium salt 	96-hour LC50: greater than 10,000 mg/L

Given the high-calculated LC50 values from the ECOSAR model, the experimentally measured toxicity of aromatic sulfonic acid derivatives, and the difficulties inherent in acute aquatic testing with dyes, no additional testing is requested.

3.3.2 Acute Toxicity to Aquatic Invertebrates

The calculated 48-hour LC50 value for FD&C Yellow No. 6 in *daphnids* is 486.5 mg/L based on input parameters for molecular weight (452.37), water solubility (190,000 mg/L at 25 °C), and melting point (390 °C), [ECOSAR EPI Suite, 2000] indicating a low order of acute toxicity. The extensive water solubility and limited lipophilicity of FD&C Yellow No. 6 is to a large extent, a function of the presence of aromatic sulfonic acid phenolic ring substituents. The extensive studies on the ecotoxicity of aromatic sulfonic acids indicate a very low order of toxicity to aquatic invertebrates [Greim *et al.*, 1994]. An experimental 24-hour EC50 value with *Daphnia* for a stilbene sulfonic acid derivative, 2,2’-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, was greater than 100 mg/L [Greim *et al.*, 1994]. This value is consistent with calculated values.

3.3.3 Acute Toxicity to Aquatic Plants

Based on input parameters for molecular weight (452.37), water solubility (190,000 mg/L at 25 °C), and melting point (390 °C), the calculated 96-hour EC50 for FD&C Yellow No. 6 with green algae is 146,000 mg/L [ECOSAR EPI Suite, 2000] indicating a very

low order of acute toxicity. In a 96-hour algal chronic toxicity test, a sulfonic acid substituted azo dye, stimulated population growth (26.4%) compared to control (algal assay medium) [Greene and Baughman, 1996]. In fact, of the 46 dyes tested, only one, an anthraquinone dye, produced measurable toxicity. Given the high-predicted value for acute toxicity to aquatic plants and the stimulation of plant growth resulting from the addition of a structurally related azo dye in an experimental acute toxicity test, it is not recommended that additional tests be performed.

3.3.4 New Testing Required

None.

[HUMAN HEALTH TOXICITY

3.3.5 Acute Toxicity

The low acute oral toxicity of FD&C Yellow No. 6 is reflected by LD50 values greater than 2,000 mg/kg [Lu and Lavalle, 1964] and 10,000 mg/kg [Gaunt *et al*., 1967] in rats, and greater than 6,000 mg/kg in mice [Gaunt *et al*., 1967].

In a pre-GLP acute toxicity study, adult male Wistar rats were administered 2000 mg/kg bw of FD&C Yellow No. 6 *via* stomach tube. The oral LD50 for FD&C Yellow No. 6 was determined to be greater than 2000 mg/kg bw [Lu and Lavalle, 1964].

In another pre-GLP acute toxicity study, groups of five male and female rats each were administered the FD&C Yellow No. 6 in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors. No deaths at up to 10,000 mg/kg bw. Slight diarrhea reported for 24 hours following treatment. Feces and urine were colored orange. No macroscopic changes reported upon necropsy. The oral LD50 for FD&C Yellow No. 6 was determined to be greater than 2000 mg/kg bw [Gaunt *et al*., 1967].

Groups of five male and female mice each (body weights: 20-25 kg) were administered the test substance in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors. No deaths at up to 6000 mg/kg bw Slight diarrhea reported for 24 hours following treatment. Feces and urine were colored orange. No macroscopic changes reported upon necropsy. The oral LD50 for FD&C Yellow No. 6 in mice was determined to be greater than 6000 mg/kg bw [Gaunt *et al*., 1967].

Groups of five male and female rats each (body weights: males 200-250 g; females 150-200 g) were administered FD&C Yellow No. 6 in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors. Slight diarrhea reported for 24

hours following treatment. Skin, feces and urine were colored orange. No macroscopic changes reported upon necropsy. The oral LD50 for FD&C Yellow No. 6 was determined to be greater than 3800 mg/kg bw [Gaunt *et al*., 1967].

Groups of five male and female mice each (body weights: 20-25 kg) were administered FD&C Yellow No. 6 in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors. Slight diarrhea reported for 24 hours following treatment. Skin, feces and urine were colored orange. No macroscopic changes reported upon necropsy. The oral LD50 for FD&C Yellow No. 6 was determined to be greater than 5500 mg/kg bw [Gaunt *et al.*, 1967].

3.3.6 *In vitro* and *In vivo* Genotoxicity

3.3.6.1 In vitro

FD&C Yellow No. 6 tested negative in reverse mutation assay using TA1535, TA1537, TA98, TA100; TA92 and TA94 with and without metabolic activation [Chung *et al*., 1981; Ishidate *et al*., 1984; Muzzall and Cook, 1979]. In one chromosomal aberration test, FD&C Yellow No. 6 tested positive at concentrations up to 6,000 micrograms/mL without metabolic activation [Ishidate *et al*., 1984], but tested negative in another chromosomal aberration test at a concentration up to 5,000 micrograms/mL with and without metabolic activation [Ivett *et al.*, 1989]. FD&C Yellow No. 6 gave a response judged to be equivocal in the sister chromatid exchange assay (SCE) at concentrations up to 5,000 micrograms/ml [Ivett *et al*., 1989].

3.3.6.2 In vivo

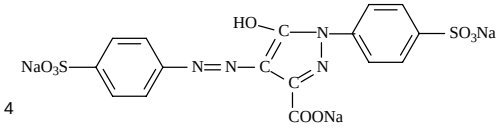
In a rodent micronucleus test, 10 ml/kg bw male rats were administered a single oral dose of 500 or 1000 mg/kg of FD&C Yellow No. 6. Bone marrow samples were taken at 24 and 48 hours later. There was no significant increase in the frequency of micronucleated polychromatic erythrocytes at either time point in either species. There was also no reported increase polychromatic erthyrocytes [Westmoreland and Gatehouse, 1991].

In an in vivo UDS assay, six to eight male Sprague-Dawley rats weighing 200-300 g were administered 500 mg/kg bw of the structurally related dye FD&C Yellow No. 5[4] *via* gavage. FD&C Yellow No. 5 did not induce unscheduled DNA synthesis at the dose level tested [Kornbrust and Barfknecht, 1985].

3.3.7 Repeat Dose Toxicity

Groups of ten male and ten female mice each were administered 0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm FD & C Yellow No. 6 in the diet daily for 12 weeks followed by one week of control diet only. Animals were housed five per cage and fed the test diet *ad libitum.* The animals were observed twice per day and weighed weekly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Mean body weight gain was decreased compared to controls among male mice receiving the 100,000 ppm intake level. Decreases in body weight gain were also reported for female mice at all intake levels, and was dose related from 12,500 ppm to 100,000 ppm. Gross and histopathological examinations revealed no treatment related lesions in male or female mice at any intake level. The NOAEL's were reported to be 50,000 ppm and less than 6,000 ppm for male and female mice, respectively [NTP, 1981].

Groups of ten male and ten female rats each were administered 0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm FD & C Yellow No. 6 in the diet daily for 12 weeks followed by one week of control diet only. Animals were housed five per cage and fed the test diet *ad libitum.* The animals were observed twice per day and weighed weekly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. No animals died during the study. Decreases in mean body weight gain were reported for male rats at the 25,000, 50,000 or 100,000 ppm intake levels. For female rats, decreases in mean body weight gain were reported at the 12,500, 25,000, 50,000 or 100,000 ppm intake levels. Bone marrow hyperplasia was reported in all examined



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animals at the 50,000 or 100,000 ppm intake levels. The NOAEL's were reported to be 6000 ppm for female rats and 12,500 ppm for male rats [NTP, 1981].

Groups of fifty male and fifty female mice each were administered 12,500 or 50,000 ppm FD & C Yellow No. 6 in the diet daily for 103 weeks. Fifty male and female mice each served as concurrent controls. Animals were housed five per cage and fed the test diet *ad libitum*. The animals were observed twice per day and weighed at least monthly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Tissues examined included adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, lymph nodes, pancreas, parathyroids, pituitary gland, rectum, skin, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder. The mean body weights of male and female mice administered the high dose were slightly lower than the control animals throughout most of the study. The survival of male and female mice was similar between treated animals and controls (males: control 38/50 (76%); low dose 40/50 (80%); and high dose 33/50 (66%) and females: control 38/50 (76%); low dose 35/50 (70%) and high dose 43/50 (86%)). An increased incidence in hepatocellular carcinomas was reported among males in the low (46%) and high (32%) dose groups compared to the control males (26%), but was only a significant difference in the low dose mice. No significant differences were observed in the female animals. The increased incidence in hepatocellular carcinomas reported for male mice was not considered clearly related to administration of the test material given the variability in tumor occurrence in control male B6C3F1 mice and because the incidence of these tumors was not significantly increased in the high dose male mice. The authors reported that under the conditions of the bioassay, there was no clear evidence of carcinogenicity of FD&C Yellow No. 6 in B6C3F1 mice [NTP, 1981].

Groups of fifty male and fifty female rats each were administered 12,500 or 50,000 ppm FD & C Yellow No. 6 in the diet daily for 103 weeks. Ninety male and female rats each served as concurrent controls. Animals were housed five per cage and fed the test diet *ad libitum*. The animals were observed twice per day and weighed at least monthly.

Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Tissues examined included the adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, lymph nodes, pancreas, parathyroids, pituitary gland, rectum, skin, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder. The mean body weights of male rats administered the high dose were slightly lower than the control animals throughout the study. The survival of male and female rats was similar between treated animals and controls (males: control 70/90 (78%); low dose 36/50 (72%); and high dose 38/50 (76%) and females: control 66/88 (75%); low dose 40/50 (80%) and high dose 37/50 (74%)). Histopathological examination revealed no evidence of carcinogenicity related to treatment with the test material. No other effects were reported. The authors reported that under the conditions of the bioassay, there was no clear evidence of carcinogenicity of FD&C Yellow No. 6 in F344/N rats [NTP, 1981].

3.3.8 Developmental Toxicity

FD&C Yellow No. 6 was administered to 140 Charles River CD rats by gavage at dose levels of 100, 300, or 1000 mg/kg bw/day. Three negative control groups (20/group) received 0.5% methocel and one positive control group (20 rats) received 7.5% retinoic acid. All females were dosed on days 6-15 of gestation. No teratogenic effects were observed in the offspring of rats receiving up to 1000 mg/kg bw/day [International Research and Development Corporation, 1972].

3.3.9 Reproductive Toxicity

In a three-generation reproduction study, 150 Charles River CD rats (10 males and 20 females/group/generation) received FD&C Yellow No. 6 at dietary levels of 0, 5, 50, 150, or 500 mg/kg/day. No treatment-related effects were observed in the parental rats or the pups receiving oral doses of up to 500 mg/kg bw/day [International Research and Development Corporation, 1974].

New Testing Required

None.

3.4 TEST PLAN TABLE

Chemical	Physical-Chemical Properties				
	Melting Point	**Boiling Point**	**Vapor Pressure**	**Partition Coefficient**	**Water Solubility**
Sunset Yellow **CAS No. 2783-94-0**	A	Calc	Calc	Calc	A

Chemical	Environmental Fate and Pathways			
	Photodegradation	**Stability in Water**	**Biodegradation**	**Fugacity**
Sunset Yellow **CAS No. 2783-94-0**	R, Calc	NA	R, Calc	Calc

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	**Acute Toxicity to Aquatic Invertebrates**	**Acute Toxicity to Aquatic Plants**
Sunset Yellow **CAS No. 2783-94-0**	R, Calc	R, Calc	R, Calc

Chemical	Human Health Data					
	Acute Toxicity	**Genetic Toxicity *In Vitro***	**Genetic Toxicity *In Vivo***	**Repeat Dose Toxicity**	**Repro-ductive Toxicity**	**Develop-mental Toxicity**
Sunset Yellow **CAS No. 2783-94-0**	A	A	A	A	A	A

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties

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201-15138B

Robust Summaries for

Sunset Yellow

CAS No. 2783-94-0

Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:
The International Association of Color Manufacturers/HPV Committee
1620 I Street, NW, Suite 925
Washington, DC 20006
Phone: 202-331-2325
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Robust Summaries

for Sunset Yellow

The evaluation of the quality of the following data uses a systematic approach described by Klimisch [Klimisch *et al*., 1996]. Based on criteria relating to international testing standards for categorizing data reliability, four reliability categories have been established. The following categories are:

- Reliability code 1. Reliable without restrictions
- Reliability code 2. Reliable with restrictions
- Reliability code 3. Not reliable
- Reliability code 4. Not assignable

1 CHEMICAL AND PHYSICAL PROPERTIES

1.1 MELTING POINT

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for substance	FD&C Yellow 6; 91.9% purity
Method/guideline	Measured
GLP	Yes
Year	1981
Remarks for Test Conditions	
Melting Point	
Decomposition	390 °C
Sublimation	
Remarks for Results	Decomposes without melting; decomposition begins at 390 °C

Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	NTP (1981) National Toxicology Program. Carcinogenesis Bioassay of FD & C Yellow No. 6. NTP 80-33.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for substance	FD&C Yellow 6
Method/guideline	Calculated
GLP	
Year	
Remarks for Test Conditions	
Melting Point	350 °C
Decomposition	
Sublimation	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI Suite (2000) US Environmental Protection Agency.

1.2 BOILING POINT

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow 6

Method/guideline	Calculated
GLP	
Year	
Remarks for Test Conditions	
Boiling Point	837 °C
Pressure	
Pressure Unit	
Decomposition	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI Suite (2000) US Environmental Protection Agency.

1.3 VAPOR PRESSURE

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for substance	FD&C Yellow 6
Method/guideline	Calculated/Mean of Antoine & Grain
GLP	No
Year	
Remarks for Test Conditions	
Vapor Pressure	1.43 X 10-22 mm Hg
Temperature	25 °C
Decomposition	

Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI Suite (2000) US Environmental Protection Agency.

1.4 N-OCTANOL/WATER PARTITION COEFFICIENTS

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for substance	FD&C Yellow No. 6
Method/guideline	Calculated
GLP	
Year	
Remarks for Test Conditions	
Log Pow	-1.18
Temperature	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	KOWWIN EPI Suite (2000) US Environmental Protection Agency.

1.5 WATER SOLUBILITY

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Purity not given
Method/guideline	Experimental
GLP	Ambiguous
Year	1991
Remarks for Test Conditions	Not given
Value (mg/L) at temperature	190,000 mg/L at 2 °C, 190,000 mg/L at 25 °C, and 200,000 mg/L at 60 °C
Description of Solubility	Not given
pH value and concentration at temp	
pKa value at 25 Celsius	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Marmion D.M. (1991) Handbook of U.S. Colorants: Foods, Drugs, and Cosmetics and Medical Devices. 3rd Ed. New York, John Wiley & Sons, Inc.

2 ENVIRONMENTAL FATE AND PATHWAYS

2.1 PHOTODEGRADATION

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Data are for structurally related sulfonic acid, 2-naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt (FD&C Red 40)
Method/guideline	Not given
Test Type	Experimental
GLP	Ambiguous
Year	1991
Light Source	15-watt General Electric germicidal lamps
Light Spectrum (nm)	Ultraviolet
Relative Intensity	
Spectrum of Substance	
Remarks for Test Conditions	The concentration of the dye solution was measured before and after the photolysis using the Hewlett-Packard 8452A diode-array UV/Visible Spectrophotometer. Red 40 was prepared in an initial concentration of 5 mg/l. In the first part of the study, photolysis experiments were conducted using two 15-W (30 Watts total) General Electric germicidal lamps as the ultraviolet light source. The distance between the light source and the reaction vessels was approximately 2.5 cm. Both direct photolysis and indirect photolysis experiments were conducted. The indirect photolysis experiment used acetone as the sensitizer for indirect photodegradation.
Concentration of Substance	5 mg/L
Temperature	
Direct photolysis	7% degradation after 50 minutes
Halflife t1/2	
Degradation % after	
Quantum yield	
Indirect photolysis	99% degradation after 20 minutes
Sensitizer	Acetone

Concentration of sensitizer	5 mg/L
Rate constant	
Degradation %after	
Breakdown products	
Remarks field for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Pasin B. and Rickabaugh J. (1991) Destruction of Azo Dyes by Sensitized Photolysis. Hazard. Ind. Wastes, 359-367.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow 6
Method/guideline	Calculation
Test Type	AOPWIN
GLP	
Year	
Light Source	
Light Spectrum (nm)	
Relative Intensity	
Spectrum of Substance	
Remarks for Test Conditions	
Concentration of Substance	
Temperature	
Direct photolysis	
Halflife t1/2	31.9 hours
Degradation % after	
Quantum yield	
Indirect photolysis	

Sensitizer	
Concentration of sensitizer	
Rate constant	
Degradation %after	
Breakdown products	
Remarks field for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	AOPWIN EPI Suite (2000) US Environmental Protection Agency.

2.2 BIODEGRADATION

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Data are for structurally related substance C.I. Acid Red No. 14
Method	Not given
Test Type	
GLP	Ambiguous
Year	1993
Contact time (units)	24 hour
Innoculum	Activated sludge
Remarks for Test Conditions	Screened raw wastewater was used as the influent in three pilot scale activated sludge biological treatment systems. Each water soluble dye was tested at doses of 1 mg/L for low spike systems and 5 mg/L for high spike systems of influent flow. Before the data collection, dye analytical recovery studies were conducted by dosing the purified dye compound into organic free water, influent wastewater, and mixed liquor. These studies were run in duplicate and each recovery study was

	repeated at least once to ensure that the dye compound could be extracted. Purified dye standards were analytically prepared from the commercial dye product by repeated recrystallization. The INF, primary effluent (PE), and ASE were filtered and the filtrate was passed through a column packed with resin. The filter paper and resin were soaked in an ammonia acetonitrile solution and then Soxhlet extracted with ammonia-acetonitrile. The extract was concentrated and brought up to 50 mL volume with a methanol/dimethylformamide solution. The mixed liquor samples were separated into two components, the filtrate or soluble fraction (SOL) and the residue (RES) fraction. The SOL fraction was processed similar to these samples but he resin adsorption step was omitted. All extracted samples were analyzed by HPLC with and ultraviolet-visible detector. Total suspended solids analyses were also performed on the INF, PE, ML, and ASE samples. All systems were operated for at least three times the solids retention time to ensure acclimation prior to initiation of data collection. All samples were 24 hr. composites made up of 6 grab samples collected every 4 hr. and stored at 4 degrees Celsius.
Degradation % after time	
Results	Percent recovery as measured: Organic Free Water: 101% at 1 mg/L and 90% at 5 mg/L; Wastewater: 98% at 1mg/L and 97% at 5 mg/L; Mixed Liquor: 88% at 1mg/L and 92% at 5 mg/L Mass Balance Data Summary: Low spike: 116% recovered, 1% adsorbed; High spike: 148% recovered, less than 1% adsorbed.
Kinetic	
Time required for 10% degradation **10 day window criteria**	
Total degradation	
Classification	
Breakdown products (transient or stable?)	
Remarks fields for results	Since the majority of the test substance was recovered, the authors assumed that this compound was not biodegraded. The authors based this assumption on preliminary data indicating little or no problems in recovering the compounds from the sample matrix. Additionally, the results also indicate that the material was not adsorbed. The authors attributed the high sulfonic acid substitution on the test substance as the reason why the material was not removed by the microbial cells or cell byproducts and subject to aerobic biodegradation.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.

Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Shaul G.M., Holdsworth T.J., Dempsey C.R., and Dostal K.A. (1990) Fate of water soluble azo dyes in the activated sludge process. Chemosphere 22, p107-119.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD & C Yellow 6
Method	BIOWIN
Test Type	Calculated
GLP	
Year	
Contact time (units)	
Innoculum	
Remarks for Test Conditions	
Degradation % after time	
Results	
Kinetic	
Time required for 10% degradation **10 day window criteria**	
Total degradation	
Classification	Not readily biodegradable
Breakdown products (transient or stable?) **Remarks fields for results**	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	BIOWIN EPI Suite (2000) US Environmental Protection Agency.

2.3 FUGACITY

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Air
absorption coefficient	
Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration	0.00219%
Remarks	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	EPIWIN EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Model Conditions	25 C, 100,000 lbs.

Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Soil
absorption coefficient	
Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration	50.1%
Remarks	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	EPIWIN EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Water

absorption coefficient	
Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration Remarks	49.8%
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	EPIWIN EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Sediment
absorption coefficient	
Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration Remarks	0.0918%
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.

Remarks for Data Reliability	Code 4. Calculated.
References	EPIWIN EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.

3 ECOTOXICITY

3.1 ACUTE TOXICITY TO FISH

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Data are for sulfonic acid derivative, 2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid
Method/guideline	
Test Type	Experimental
GLP	Ambiguous
Year	Not given
Species/Strain/Supplier	Fish
Analytical monitoring	
Exposure period (unit)	48 hour
Remarks for Test Conditions	
Observations on precipitation	
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
Endpoint value	LC50 = 200 mg/L
Reference substances (if used)	
Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Alstoffe, (1992) Daten zur Beurteilung der Wirkung auf Mensch and Umwelt-Satensatze, Verband der Chemischen Industrie, Frankfurt 1992. Schön N. (1991) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 3(3), 183-185.

	Schön N. (1992) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 4(6), 343-345.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Data are for sulfonic acid derivative,2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, disodium salt
Method/guideline	
Test Type	Experimental
GLP	Ambiguous
Year	Not given
Species/Strain/Supplier	Fish
Analytical monitoring	
Exposure period (unit)	72 hour
Remarks for Test Conditions	
Observations on precipitation **Nominal concentrations as mg/L** **Measured concentrations as mg/L** **Unit**	
Endpoint value	LC50 greater than 1000 mg/L
Reference substances (if used) **Remarks fields for results**	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Alstoffe, (1992) Daten zur Beurteilung der Wirkung auf Mensch and Umwelt-Satensatze, Verband der Chemischen Industrie, Frankfurt 1992. Schön N. (1991) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 3(3), 183-185. Schön N. (1992) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 4(6), 343-345.

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Data are for sulfonic acid derivative, 2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, dipotassium salt
Method/guideline	
Test Type	Experimental
GLP	Ambiguous
Year	Not given
Species/Strain/Supplier	Fish
Analytical monitoring	
Exposure period (unit)	96 hour
Remarks for Test Conditions	
Observations on precipitation **Nominal concentrations as mg/L** **Measured concentrations as mg/L** **Unit**	
Endpoint value	LC50 greater than 10000 mg/L
Reference substances (if used) **Remarks fields for results**	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Alstoffe, (1992) Daten zur Beurteilung der Wirkung auf Mensch and Umwelt-Satensatze, Verband der Chemischen Industrie, Frankfurt 1992. Schön N. (1991) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 3(3), 183-185. Schön N. (1992) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 4(6), 343-345.

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow 6
Method/guideline	ECOSAR
Test Type	Calculated
GLP	
Year	
Species/Strain/Supplier	Fish
Analytical monitoring	
Exposure period (unit)	96 hour
Remarks for Test Conditions	Input parameters: Molecular weight, Water solubility, 190,000 mg/L at 25 °C; melting point 390 °C
Observations on precipitation **Nominal concentrations as mg/L** **Measured concentrations as mg/L** **Unit**	
Endpoint value	LC50 = 6044 mg/L
Reference substances (if used) **Remarks fields for results**	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

3.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Data are for sulfonic acid derivative, 2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid
Method/guideline	
Test Type	Experimental
GLP	
Year	
Analytical procedures	
Species/Strain	*Daphnia magna*
Test details	24 hour
Remarks for Test Conditions	
Nominal concentrations as mg/L **Measured concentrations as mg/L** **Unit**	
EC50, EL50, LC0, at 24,48 hours **Biological observations**	EC50 = 100 mg/L
Control response satisfactory? **Appropriate statistical evaluations?** **Remarks fields for results**	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Alstoffe, (1992) Daten zur Beurteilung der Wirkung auf Mensch and Umwelt-Satensatze, Verband der Chemischen Industrie, Frankfurt 1992. Schön N. (1991) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 3(3), 183-185. Schön N. (1992) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 4(6), 343-345.

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow 6
Method/guideline	ECOSAR
Test Type	Calculated
GLP	
Year	
Analytical procedures	
Species/Strain	*Daphnia magna*
Test details	48 hours
Remarks for Test Conditions	Input parameters: Water solubility, 190,000 mg/L at 25 °C; Molecular weight 452.37; Melting point 390 °C
Nominal concentrations as mg/L **Measured concentrations as mg/L** **Unit**	
EC50, EL50, LC0, at 24,48 hours **Biological observations**	EC50 = 486.5 mg/L
Control response satisfactory? **Appropriate statistical evaluations?** **Remarks fields for results**	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

3.3 ACUTE TOXICITY TO AQUATIC PLANTS

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	The test substance was an unidentified sulfonic acid substituted azo dye.
Method/guideline	
Test Type	Experimental
GLP	Ambiguous
Year	1996
Species/Strain/Supplier	Green algae, *Selenatrum capricornutum*
Endpoint basis	
Exposure period (duration)	96 hour
Analytical monitoring	
Remarks for Test Conditions	Algal chronic toxicity test were performed according the method of EPA, 1988. Three replicates were performed for each dye at a nominal concentration of 1 mg/l for the active colorant. One ml of dye stock solution was added to 50 mg/l of algal assay medium in 125 ml Erlenmeyer flasks. S. capricornutum in continuous culture provided the initial innoculum (10,000 algal cells/ml). The cells were incubated in the solution for 96 hours. The diluent and negative control were algal assay medium. AAM was prepared by adding 1 ml from each of five stock solutions to 900 ml of deionized water. After spiking, the total volume was brought to 1 liter with deionized water. Population growth was used to establish potential toxicity. If the dye inhibited algal growth by more than 50% of that of the negative controls, a definitive test using several dilutions of the dye was performed to allow for determination of an EC50 concentration.
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
Endpoint value	Average yield: 36.6% with 95% C.I. (34.9-38.4).
NOEC, LOEC or NOEL, LOEL	
Biological observations	26.4% stimulation of population growth compared to control.
Control response satisfactory?	Yes
Appropriate statistical evaluations?	Yes, Dunnett's test
Remarks fields for results	Not statistically significant.

Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Greene J. C. and Baughman G.L. (1996) Effects of 46 dyes on population-growth of fresh-water green-alga selenastrum-capricornutum. Textile Chemist And Colorist, 28, 23-30. Green J.D. et al. (1988) Protocols for short term toxicity screening of hazardous w
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow 6
Method/guideline	ECOSAR
Test Type	Calculated
GLP	
Year	
Species/Strain/Supplier	Green algae
Endpoint basis	
Exposure period (duration)	96 hour
Analytical monitoring	
Remarks for Test Conditions	Input parameters: Water solubility - 190,000 mg/L at 25 °C; Molecular weight 452.37; Melting point 390 °C
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
Endpoint value	EC50 = 146,000 mg/L
NOEC, LOEC or NOEL, LOEL	
Biological observations	
Control response satisfactory?	
Appropriate statistical evaluations?	
Remarks fields for results	
Conclusion remarks	

Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) US Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

4 HUMAN HEALTH TOXICITY

4.1 ACUTE TOXICITY

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Not given
Method/guideline	Not given
Test Type	Acute Toxicity LD50
GLP	No
Year	1964
Species/Strain	Rats/Wistar
Sex	Male
# of animals per sex per dose	6
Vehicle	Water
Route of administration	Oral-Gavage
Remarks for test conditions	Wistar adult male rats were administered 2000 mg/kg bw *via* stomach tube.
Value LD50 or LC50 with confidence limits	Greater than 2000 mg/kg bw
Number of deaths at each dose level	0 deaths
Remarks for results	
Conclusion remarks	The oral LD50 for sunset yellow is greater than 2000 mg/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Lu F. and Lavalle C. (1964) The acute toxicity of some synthetic colours used in drugs and foods. Canadian Pharmaceutical Journal 9.

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Greater than 85% purity
Method/guideline	LD50 calculated by Weil (1952)

Test Type	Acute Toxicity LD50
GLP	No
Year	1967
Species/Strain	Rats/Carworth Farm E strain
Sex	Male and Female
# of animals per sex per dose	5
Vehicle	Water
Route of administration	Oral
Remarks for test conditions	Groups of five male and female rats each (body weights: males 200-250 g; females 150-200 g) were administered the test substance in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors.
Value LD50 or LC50 with confidence limits	Greater than 10,000 mg/kg
Number of deaths at each dose level	No deaths at up to 10,000 mg/kg bw.
Remarks for results	Slight diarrhea reported for 24 hours following treatment. Feces and urine were colored orange. No macroscopic changes reported upon necropsy.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Gaunt I.F., Farmer M., Grasso P., and Gangolli .D. (1967) Acute (Rat and Mouse) and Short-term (Rat) Toxicity Studies on Sunset Yellow FCF. Fd Cosmet Toxicol 5, pp. 747-754.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Greater than 85% purity
Method/guideline	LD50 calculated by Weil (1952)
Test Type	Acute Toxicity LD50
GLP	No
Year	1967
Species/Strain	Mice/ICI Alderley Park strain
Sex	Male and Female

# of animals per sex per dose	5
Vehicle	Water
Route of administration	Oral
Remarks for test conditions	Groups of five male and female mice each (body weights: 20-25 kg) were administered the test substance in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors.
Value LD50 or LC50 with confidence limits	Greater than 6000 mg/kg bw
Number of deaths at each dose level	No deaths at up to 6000 mg/kg bw
Remarks for results	Slight diarrhea reported for 24 hours following treatment. Feces and urine were colored orange. No macroscopic changes reported upon necropsy.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Gaunt I.F., Farmer M., Grasso P., and Gangolli .D. (1967) Acute (Rat and Mouse) and Short-term (Rat) Toxicity Studies on Sunset Yellow FCF. Fd Cosmet Toxicol 5, pp. 747-754.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Greater than 85% purity
Method/guideline	LD50 calculated by Weil (1952)
Test Type	Acute Toxicity LD50
GLP	No
Year	1967
Species/Strain	Rats/Carworth Farm E strain
Sex	Male and Female
# of animals per sex per dose	5
Vehicle	Water
Route of administration	Intraperitoneal
Remarks for test conditions	Groups of five male and female rats each (body weights: males 200-250 g; females 150-200 g) were administered the test substance in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died

	and some survivors.
Value LD50 or LC50 with confidence limits	3800 mg/kg bw (2900-4600 mg/kg bw)
Number of deaths at each dose level	Not given
Remarks for results	Slight diarrhea reported for 24 hours following treatment. Skin, feces and urine were colored orange. Deaths were preceded by comas, and in some animals convulsions. No macroscopic changes reported upon necropsy.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Gaunt I.F., Farmer M., Grasso P., and Gangolli .D. (1967) Acute (Rat and Mouse) and Short-term (Rat) Toxicity Studies on Sunset Yellow FCF. Fd Cosmet Toxicol 5, pp. 747-754.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	Greater than 85% purity
Method/guideline	LD50 calculated by Weil (1952)
Test Type	Acute Toxicity LD50
GLP	No
Year	1967
Species/Strain	Mice/ICI Alderley Park strain
Sex	Male and Female
# of animals per sex per dose	5
Vehicle	Water
Route of administration	Intraperitoneal
Remarks for test conditions	Groups of five male and female mice each (body weights: 20-25 kg) were administered the test substance in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors.
Value LD50 or LC50 with confidence limits	5500 (95% C.I.: 4600-6700) mg/kg bw (Males) 4600 (95% C.I.: 3900-5300) (Females)
Number of deaths at each dose level	Not given
Remarks for results	Slight diarrhea reported for 24 hours following treatment. Skin, feces and urine were colored orange. Deaths were preceded by comas, and in some animals convulsions. No macroscopic

	changes reported upon necropsy.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Gaunt I.F., Farmer M., Grasso P., and Gangolli .D. (1967) Acute (Rat and Mouse) and Short-term (Rat) Toxicity Studies on Sunset Yellow FCF. Fd Cosmet Toxicol 5, pp. 747-754.

4.2 GENETIC TOXICITY

4.2.1 In vitro Genotoxicity

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6; Purity not given
Method/guideline	Ames plate incorporation and liquid pre-incubation
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1981
Species/Strain	*Salmonella typhimurium* TA1535, TA 1537, TA1538, TA98, TA100
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats
Doses/concentration levels	.005- 5.0 mg/plate
Statistical Methods	Not given
Remarks for test conditions	Reverse mutation tests were carried out using S. typhimurium strains TA1535, TA 1537, TA1538, TA98, TA100. Plate incorporation tests were conducted according to Ames et al., with the Andrews et al. modifications. Duplicates were performed at each of the six concentrations of the test substance. Mutagenic compounds were assayed using duplicate plates. A substance was considered positive when

	the number of revertants above background was at least twice the value of the historical control mean or twice the value of the current control mean, whichever was greater and a dose response curve could be generated. Positive controls without metabolic activation were sodium azide (TA1535 and TA100), 9-aminoacridine (TA97 and TA1535), and 4-nitro-o-phenylenediamine (TA98). The positive controls were sodium azide, 9-aminoacridine, 2-nitrofluorene, and 2-aminoanthracene.
Result	Negative
Cytotoxic concentration	5.0 mg/plate for plate-incorporation, and .5 mg/ml for pre-incubation test
Genotoxic effects	Negative
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion remarks	The test substance was negative in the AMES assay for reverse mutation using Salmonella typhimurium TA1535, TA 1537, TA1538, TA98, TA100.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	Chung K.T., Fulk G.E., & Andrews A.W. (1981) Mutagenicity testing of some commonly used dyes. Applied and Environmental Microbiology 42, 641-648.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6; Purity not given
Method/guideline	Ames, McCann and Yamasaki (1975)
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1984
Species/Strain	*Salmonella typhimurium* TA1535, TA 1537, TA98, TA100, TA92, TA94
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats
Doses/concentration levels	up to 5.0 mg/ml
Statistical Methods	Not given
Remarks for test conditions	Reverse mutation tests were carried out using S. typhimurium strains TA92, TA1535, TA100, TA1537, TA94 and TA98. Cells

	cultured overnight were pre-incubated with the test substance and the S-9 mix for twenty minutes at 37 degrees Celsius prior to plating. Duplicates were performed at each of the six concentrations of the test substance. The number of revertant colonies were counted following incubation for two days. Negative controls were either untreated plates or solvent. Positive results were determined if the number of colonies found was twice the number in the control. If the test was positive and a dose response relationship was not detected, additional experiments at different doses or induced mutation frequency assays were performed.
Result	Negative
Cytotoxic concentration	5.0 mg/ml was the highest non-cytotoxic dose used in the experiment.
Genotoxic effects	Negative
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion remarks	Sunset Yellow was negative in the AMES assa;y for reverse mutation using Salmonella typhimurium TA1535, TA 1537, TA98, TA100, TA92, TA94.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report which meets basic scientific principles.
References	Ishidate, M., Sofuni, T., Yoshikawa, K., Hauashi, M., Nohmi, T., Sawada, M. and Matsuoka. (1984). Primary Mutagenicity Screening of Food Additives Currently Used in Japan. Fd. Chem. Toxic. 22(8) 623-636.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6; Purity not given
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	No
Year	1979
Species/Strain	*Salmonella typhimurium* TA1535, TA 1537, TA98, TA100
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats
Doses/concentration levels	10-250 mg/plate
Statistical Methods	Not given

Remarks for test conditions	The test substance was dissolved in DMSO. The test was considered positive if 2 fold increase in revertants was observed. Positive controls included 9-aminoacridine; 2-aminoflourine; and N-methyl-N-nitrosoguanidine.
Result	Negative
Cytotoxic concentration	Not given
Genotoxic effects	Negative
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion remarks	No evidence of genotoxicity was reported.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Muzzall J.M. and Cook W.I. (1979) Mutagenicity test of dyes used in cosmetics with the Salmonella/mammalian microsome test. Mutations Research 67, 1-8.a
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6; Purity 91.8%
Method/guideline	Sister Chromatid Exchange test was carried out using a Chinese hamster ovary (CHO).
Test Type	Sister Chromatid Exchange
System of Testing	Chinese hamster ovary cells
GLP	Ambiguous
Year	1989
Species/Strain	Chinese hamster ovary cells (CHO)
Metabolic Activation	With and without metabolic activation
Doses/concentration levels	up to 5,000 micrograms/mL
Statistical Methods	Trend test.
Remarks for test conditions	Sister chromatid exchange tests were carried out using the Chinese hamster ovary cells. Cells were exposed to the test substance for 25 hr. With metabolic activation, the cells were exposed to the test chemical plus the metabolic activation for 2 hr. For both tests (with and without metabolic activation) 10 micromolar bromodeoxyuridine (BrdUrd) was added 2 hours following initiation of the test. Colcemid was present for the last 2-2.5 hours of the incubation. Without metabolic activation, the total incubation time was 27.5-28 hr and the cells were washed

	prior to the addition of the Colcemid. The cultures with metabolic activation were washed to remove the test substance and the metabolic activation 2 hours following initial exposure.
Result	In one trial without activation, SCE's were induced at 30 and 25% respectively at 1,667 and 5,000 micrograms/ml. With activation, the test substance did not induce SCE's at concentrations up to 5000 micrograms/mL.
Cytotoxic concentration	Not given
Genotoxic effects	Equivocal.
Appropriate statistical evaluations?	Yes, trend test
Remarks for results	Equivocal without activation. Negative with activation.
Conclusion remarks	The SCE response to FD&C Yellow No. 6 was judged to equivocal without activation and negative with activation.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report which meets basic scientific principles.
References	Ivett J.L., Brown B.M., Rodgers C., Anderson B.E., Resnick M.A., and Zeigler, E. (1989) Chromosomal aberrations and sister chromatid exchange tests in Chinese Hamster Ovary Cells in Vitro. IV. Results with 15 chemicals. Environmental and Molecular Mut
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6; Purity 91.8%
Method/guideline	Chromosomal aberration test was carried out using a Chinese hamster ovary cell line, CHL.
Test Type	Chromosomal aberration test
System of Testing	Chinese hamster ovary cells
GLP	Ambiguous
Year	1989
Species/Strain	Chinese hamster ovary cells (CHO)
Metabolic Activation	With and without metabolic activation
Doses/concentration levels	up to 5,000 micrograms/L
Statistical Methods	
Remarks for test conditions	Chromosomal aberration tests were carried out using the Chinese hamster ovary cells. Cells were exposed to the test substance for 8 hr. With metabolic activation, the cells were exposed to the test chemical plus the metabolic activation for 2 hr, washed, incubated for 8 hr., and then treated with Colcemid for 2-2.5 hr. The cells were prepared for viewing on slides.

Result	Negative with and without metabolic activation.
Cytotoxic concentration	Not given
Genotoxic effects	Negative
Appropriate statistical evaluations?	Yes, trend test
Remarks for results	Negative
Conclusion remarks	Sunset Yellow tested negative in the chromosomal aberration test using Chinese hamster ovary cells.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report which meets basic scientific principles.
References	Ivett J.L., Brown B.M., Rodgers C., Anderson B.E., Resnick M.A., and Zeigler, E. (1989) Chromosomal aberrations and sister chromatid exchange tests in Chinese Hamster Ovary Cells in Vitro. IV. Results with 15 chemicals. Environmental and Molecular Mut
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6; Purity not given
Method/guideline	Chromosomal aberration test was carried out using a Chinese hamster fibroblast cell line, CHL. The cells were exposed to 3 different doses for 24 and 48 hours. No metabolic activation system was applied.
Test Type	Chromosomal aberration test
System of Testing	Chinese hamster fibroblast cell line CHL.
GLP	Ambiguous
Year	1984
Species/Strain	Chinese hamster fibroblast cell line CHL.
Metabolic Activation	None
Doses/concentration levels	up to 6.0 mg/ml
Statistical Methods	
Remarks for test conditions	Chromosomal aberration tests were carried out using the Chinese hamster fibroblast line. Cells were exposed to the test substance at three different doses for 24 and 48 hr. No metabolic activation was employed. The maximum dose used for each test substance was found in a preliminary test to determine the dose required for 50% cell-growth inhibition. Colcemid at a final concentration of 0.2 ug/ml was added to the culture two hours prior to cell harvesting. The cells were prepared for viewing on slides. One hundred visible

	metaphases were observed under the microscope and the incidence of polyploid cells and structural chromosomal aberrations (including choromosome and chromatid gaps, breaks, exchanges, ring formations, fragmentations and others were recorded. Negative controls included untreated cells and solvent treated cells. The incidence of aberrations in the negative controls was generally less than 3.0%. The results were considered negative if less than 4.9%, equivocal if between 5.0-9.9%, and positive if more than 10%. If dose response relationships were not observed, additional experiments were carried out at similar dose levels. The maximum dose for positive results represents the dose at which the maximum effect was obtained. For quantitative evaluation of the clastogenic potential, the D20 was calculated, which is the dose (mg/ml) at which structural aberrations (including gaps) were detected in 20% of the metaphases observed. In addition, the TR value was calculated, which indicates the frequency of cells with exchange-type aberrations per unit dose (mg/ml). These values are relatively high for chemicals that show carcinogenic potential in animals.
Result	The test substance was shown to be positive (20% total incidence of cells with aberrations) in chromosomal aberration test at 48 hours. TR value was 1.8 and D20=2.0. It was also positive at 2.0 mg/ml at 24 hour and 48 hour, (23.0 and 18%, total incidence of cells with aberrations) The results were considered positive if the total incidence of cells with aberrations (including gaps) was 10.0% or more.
Cytotoxic concentration	Not given
Genotoxic effects	Positive
Appropriate statistical evaluations?	None given
Remarks for results	Positive
Conclusion remarks	Sunset Yellow tested positive in the chromosomal aberration test using Chinese hamster fibroblasts.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report which meets basic scientific principles.
References	Ishidate, M., Sofuni, T., Yoshikawa, K., Hauashi, M., Nohmi, T., Sawada, M. and Matsuoka. (1984). Primary Mutagenicity Screening of Food Additives Currently Used in Japan. Fd. Chem. Toxic. 22(8) 623-636.

4.2.2 In vivo Genotoxicity

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Method/guideline	Rodent Micronucleus Test
Test Type	Rodent Micronucleus
GLP	Ambiguous
Year	1991
Species/Strain	Rat/PVG
Sex	Male
Route of administration	Oral-Gavage
Doses/concentration levels	10 ml/kg bw
Exposure period	Single dose
Remarks for test conditions	Male PVG rats received a single oral dose of 500, or 1000 mg/kg of the test substance. Bone marrow samples were taken at 24 and 48 hours later.
Effect on mitotic index or PCE/NCE ratio by dose level and sex	
Genotoxic effects	No significant increase in the frequency of micronucleated polychromatic erythrocytes at either time point and in either species was reported. Additionally, there was reported increase in the % PE (polychromatic erthyrocytes).
NOEL (C)/ LOEL (C)	
Appropriate statistical evaluations?	Yes.
Remarks for results	No effects.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report which meets basic scientific principles.
References	Westmoreland C. and Gatehouse D.G. (1991) The differential clastogenicity of Solvent Yellow 14 and FD & C Yellow No. 6 in vivo in the rodent micronucleus test (observations on species and tissue specificity). Carcinogenesis 12 (8), 1403-8.
CAS Numerical	2783-94-0

Substance Name	Sunset Yellow
Remarks for Substance	Data are for structurally related substance, C.I. Acid Yellow 23, 94% purity
Method/guideline	Mirsalis and Butterworth, 1980
Test Type	Unscheduled DNA Synthesis
GLP	Ambiguous
Year	1985
Species/Strain	Rat/Sprague Dawley
Sex	Male
Route of administration	Oral-Gavage
Doses/concentration levels	500 mg/kg bw
Exposure period	2 hour; 15 hour
Remarks for test conditions	Six to eight male Sprague-Dawley rats weighing 200-300 g were administered 500 mg acid yellow 23/kg bw via gavage. The control animal was administered corn oil only. Animals were killed at two timepoints, 2 hr and 15 hr. If negative results were obtained at timepoint 1 and timepoint 2, the in vivo testing was terminated and considered to be negative. If the initial test at timepoint 1 yielded a positive response, the test substance was retested at that timepoint. If another positive response was observed, the test was considered positive. Timepoints are the time the test substance was administered prior to the start of liver perfusion and isolation of hepatocytes. Hepatocytes from rats were isolated and cultured according to the two step in situ liver perfusion model (Malansky and Williams, 1982). Viable hepatocytes (2 X 10+5) were seeded in wells and incubated for 4 hours with [H3]-thymidine (10 uCi/ml) and the test substance (prepared in either DMSO or water) according to a procedure similar to Williams, 1977. Control incubations were conducted with and without DMSO. The authors state that DMSO had no effect on DNA repair. DNA repair was quantified by the autoradiographic determination of incorporated [3H]-thymidine. Net nuclear grains (NNG) were determined by counting the number of grains in each nuclei and subtracting the average number of grains present in the three equal size adjacent cytoplasmic areas. Average NNG counts of 5 or more were assumed to constitute a positive response, because these differed from the control response by greater than 2 standard deviations. In the negative controls, NNG counts ranged from -0.6- to -2.8 and from -0.9 to -2.1 for no solvent and 1% DMSO incubations, respectively. The proportion of cells with greater than or equal to 5 NNG was less than or equal to 8.1% for all control incubations. Therefore NNG below zero were considered negative responses. Concentrations of dyes producing 90% or greater detachment of the hepatocytes from the coverslips

	were assumed to be toxic and not counted.
Effect on mitotic index or PCE/NCE ratio by dose level and sex	The positive control was Solvent Yellow 3 (o-aminoazotoluene). Experiment 1 Dose (mg/kg bw) Time Avg NNG % >5NNG 500 2 hr -2.6 (+/-3.7) 2 15 hr -1.3 (+/-2.6) 2
Genotoxic effects	Negative
NOEL (C)/ LOEL (C)	Greater than 500 mg/kg bw
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion remarks	C.I. Acid Yellow 23 did not induce unscheduled DNA synthesis in an *in vivo* assay using rat hepatocytes isolated from the livers of Sprague Dawley rats.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Kornbrust D. and Barfknecht T. (1985) Testing Dyes in HPC/DR systems. Environmental Mutagenesis 7, 101-120.

4.3 REPEATED DOSE TOXICITY

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	91.9% purity; 5.05% water; 2.77% sodium chloride
Method/guideline	National Toxicology Program. Carcinogenesis bioassay NTP 80-33
GLP	Yes
Year	1981
Species/Strain	Rats/F344/N
Sex	Male and Female
Route of administration	Oral-Diet

Doses/concentration levels	0, 12,500 or 25,000 ppm
Exposure period	103 weeks
Frequency of treatment	Daily
Control Group	Yes
Post exposure observation period	1 week
Remarks for test conditions	Groups of fifty male and fifty female rats each were administered 12,500 or 50,000 ppm FD & C Yellow No. 6 in the diet daily for 103 weeks. Ninety male and female rats each served as concurrent controls. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed at least monthly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Tissues examined included adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, lymph nodes, pancreas, parathyroids, pituitary gland, rectum, skin, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder.
NOAEL(NOEL)	25,000 ppm (females); 12,500 ppm (males)
LOAEL(LOEL)	Greater than 25,000 ppm (females); 25,000 ppm (males)
Actual dose received by dose level and sex	not determined
Toxic response/effects by dose level	The mean body weights of male rats administered the high dose were slightly lower than the control animals throughout the study. The survival of male and female rats was similar between treated animals and controls (males: control 70/90 (78%); low dose 36/50 (72%); and high dose 38/50 (76%) and females: control 66/88 (75%); low dose 40/50 (80%) and high dose 37/50 (74%)). Histopathological examination revealed no evidence of carcinogenicity related to treatment with the test material. No other effects were reported.
Appropriate statistical evaluations?	Yes, Cox and Taron
Remarks for results	See Toxic response/effects by dose level.
Conclusion remarks	The authors reported that under the conditions of the bioassay, there was no clear evidence of carcinogenicity of FD & C Yellow No. 6 in F344/N rats.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	NTP (1981) National Toxicology Program. Carcinogenesis Bioassay of FD & C Yellow No. 6. NTP 80-33.
CAS Numerical	2783-94-0

Substance Name	Sunset Yellow
Remarks for Substance	91.9% purity; 5.05% water; 2.77% sodium chloride
Method/guideline	National Toxicology Program. Carcinogenesis bioassay NTP 80-33
GLP	Yes
Year	1981
Species/Strain	Mice/B6C3F1
Sex	Male and Female
Route of administration	Oral-Diet
Doses/concentration levels	0, 12,500 or 25,000 ppm
Exposure period	103 weeks
Frequency of treatment	Daily
Control Group	Yes
Post exposure observation period	1 week (female mice)
Remarks for test conditions	Groups of fifty male and fifty female mice each were administered 12,500 or 50,000 ppm FD & C Yellow No. 6 in the diet daily for 103 weeks. Fifty male and female mice each served as concurrent controls. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed at least monthly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Tissues examined included adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, lymph nodes, pancreas, parathyroids, pituitary gland, rectum, skin, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder.
NOAEL(NOEL)	12,500 ppm
LOAEL(LOEL)	25,000 ppm
Actual dose received by dose level and sex	not determined
Toxic response/effects by dose level	The mean body weights of male and female mice administered the high dose were slightly lower than the control animals throughout most of the study. The survival of male and female mice was similar between treated animals and controls (males: control 38/50 (76%); low dose 40/50 (80%); and high dose 33/50 (66%) and females: control 38/50 (76%); low dose 35/50 (70%) and high dose 43/50 (86%)). An increased incidence in hepatocellular carcinomas was reported among males in the low (46%) and high (32%) dose groups compared to the control males (26%), but was only a significant difference in the low

	dose mice. No significant differences were observed in the female animals. The increased incidence in hepatocellular carcinomas reported for male mice was not considered clearly related to administration of the test material given the variability in tumour occurrence in control male B6C3F1 mice and because the incidence of these tumours was not significantly increased in the high dose male mice.
Appropriate statistical evaluations?	Yes, Cox and Taron
Remarks for results	
Conclusion remarks	The authors reported that under the conditions of the bioassay, there was no clear evidence of carcinogenicity of FD & C Yellow No. 6 in B6C3F1 mice.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	NTP (1981) National Toxicology Program. Carcinogenesis Bioassay of FD & C Yellow No. 6. NTP 80-33.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	91.9% purity; 5.05% water; 2.77% sodium chloride
Method/guideline	12 week range finding study. National Toxicology Program. Carcinogenesis bioassay NTP 80-33
GLP	Yes
Year	1981
Species/Strain	Rat/F344/N
Sex	Male and Female
Route of administration	Oral-Diet
Doses/concentration levels	0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm
Exposure period	12 weeks
Frequency of treatment	Daily
Control Group	Yes
Post exposure observation period	1 week
Remarks for test conditions	Groups of ten male and ten female rats each were administered 0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm FD & C Yellow No. 6 in the diet daily for 12 weeks followed by one week of control diet only. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed weekly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals.

NOAEL(NOEL)	6000 ppm (females); 12,500 ppm (males)
LOAEL(LOEL)	12,500 ppm (females); 25,000 ppm (males)
Actual dose received by dose level and sex	not determined
Toxic response/effects by dose level	No animals died during the study. Decreases in mean body weight gain were reported for male rats at the 25,000, 50,000 or 100,000 ppm intake levels. For female rats, decreases in mean body weight gain were reported at the 12,500, 25,000, 50,000 or 100,000 ppm intake levels. Bone marrow hyperplasia was reported in all examined animals at the 50,000 or 100,000 ppm intake levels.
Appropriate statistical evaluations?	Yes, Cox and Taron
Remarks for results	See Toxic response/effects by dose level.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	NTP (1981) National Toxicology Program. Carcinogenesis Bioassay of FD & C Yellow No. 6. NTP 80-33.
CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	91.9% purity; 5.05% water; 2.77% sodium chloride
Method/guideline	12 week range finding study. National Toxicology Program. Carcinogenesis bioassay NTP 80-33
GLP	Yes
Year	1981
Species/Strain	Mice/B6C3F1
Sex	Male and Female
Route of administration	Oral-Diet
Doses/concentration levels	0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm
Exposure period	12 weeks
Frequency of treatment	Daily
Control Group	Yes
Post exposure observation period	1 week
Remarks for test conditions	Groups of ten male and ten female mice each were administered 0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm FD & C Yellow No. 6 in the diet daily for 12 weeks followed by

	one week of control diet only. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed weekly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals.
NOAEL(NOEL)	50,000 ppm (male); less than 6000 ppm (female)
LOAEL(LOEL)	100,000 ppm (male); 6000 ppm (female)
Actual dose received by dose level and sex	not determined
Toxic response/effects by dose level	Mean body weight gain was decreased compared to controls among male mice receiving the 100,000 ppm intake level. Decreases in body weight gain were also reported for female mice at all intake levels, and was dose related from 12,500 ppm to 100,000 ppm. Gross and histopathological examinations revealed no treatment related lesions in male or female mice at any intake level.
Appropriate statistical evaluations?	Yes, Cox and Taron
Remarks for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	NTP (1981) National Toxicology Program. Carcinogenesis Bioassay of FD & C Yellow No. 6. NTP 80-33.

4.4 DEVELOPMENTAL TOXICITY

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Method/guideline	Teratogenicity study
Test Type	
GLP	Ambiguous
Year	1974

Species/Strain	Rat/Charles River CD
Sex	Female
Route of administration	Oral-Gavage
Duration of test	20 days
Doses/concentration levels	0, 100, 300 or 1000 mg/kg bw/day
Exposure period	9 days
Frequency of treatment	Daily
Control Group and treatment	Yes, three negative control groups were maintained and administered 0.5% methocel, while one positive control group was maintained and administered 7.5% mg/kg bw/day of retinoic acid.
Remarks for test conditions	FD&C Yellow No. 6 was administered by gavage at dose levels of 100, 300 or 1000 mg/kg bw/day to 140 female Charles River CD rats. Three negative control groups (20/group) received the vehicle control while one control group received the positive control (7.5% mg/kg bw/day retinoic acid). All females were dosed on days 6-15 of gestation. Cesarean sections were performed on the 20th day of gestation.
NOAEL(NOEL) maternal toxicity	
LOAEL(LOEL) maternal toxicity	Not given
NOAEL (NOEL) developmental toxicity	100 mg/kg bw/day
LOAEL (LOEL) developmental toxicity	300 mg/kg bw/day
Actual dose received by dose level and sex	Not given
Maternal data with dose level	
Fetal data with dose level	The mean weights of the offspring from the 300 and 1000 mg/kg bw/day groups were decreased when compared to the average fetus weight of the combined negative controls. There were no compound related effects on early or late resorptions, empty implantation sites, body weight or numbers of live or dead fetuses. No teratogenicity was observed among the offspring.
Appropriate statistical evaluations?	Not given
Remarks for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	International Research and Development Corporation (1972) Teratology study in rats. Compound FD&C Yellow No. 6. Unpublished report no. 306-004.

4.5 REPRODUCTIVE TOXICITY

CAS Numerical	2783-94-0
Substance Name	Sunset Yellow
Remarks for Substance	FD&C Yellow No. 6
Method/guideline	3-generation reproductive study
Test Type	
GLP	Ambiguous
Year	1974
Species/Strain	Rat/Charles River CD
Sex	Male and Female
Route of administration	Oral-Diet
Duration of test	
Doses/concentration levels	5, 50, 150 or 500 mg/kg bw/day
Premating Exposure period for males	
Premating Exposure period for females	
Frequency of treatment	Daily
Control Group and treatment	Yes.
Remarks for test conditions	One hundred twenty Charles River CD rats (10 males and 20 females/group/generation) received 5, 50, 150 or 500 mg/kg bw/day of the test substance as a dietary admixture in a three-generation study. Ten males and twenty females received no compound and served as controls.
NOAEL(NOEL)	500 mg/kg bw/day
LOAEL(LOEL)	Not determined
Actual dose received by dose level and sex	Not given
Parental data and F1 as appropriate	

Offspring toxicity F1 and F2

Appropriate statistical evaluations?	
Remarks for results	There were no compound related effects on fertility, gestation, pup viability or lactation indices, on reproductive organs of females, or on organ weights among parents and offspring. There were no compound related lesions in any tissue examined histologically, including kidneys and adrenal glands from parental rats or from offspring.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	International Research and Development Corporation (1974) Multi-generation reproduction study in rats. Compound FD&C Yellow No. 6. Unpublished report no. 306-005.